(*Reprinted from Nature, Vol.* 181, *pp.* 1419–1420, *May* 17, 1958)

Antibody Production by Single Cells*

FAGREUS[1] and others[2,3] have shown that certain tissues from pre-sensitized animals can form antibody *in vitro*. This communication describes a technique whereby antibody production by single cells isolated in microdroplets can be detected. The technique is based on specific immobilization of ***Salmonella*** serotypes by anti-flagellar antibody. It was observed that single cells from a rat, simultaneously stimulated with two antigens, formed detectable amounts of one or the other antibody.

Two monophasic ***Salmonellae*** were used: *S. adelaide*, flagellar antigen H_1^{g}, and *S. typhi*, H_1^{d}. They were maintained at maximum motility by frequent passages through a semi-solid nutrient gelatin agar medium[4]. A formalinized broth culture containing about 10^9 organisms per ml. was used as the antigen. Adult Wistar rats were injected with 0·25 ml. of a mixture of equal parts of both antigens into both hind foot-pads. Usually the animals were given three pairs of injections at three weekly intervals. Three days after the tertiary injections, they were killed by exsanguination under anæsthesia. Both popliteal lymph nodes were removed, pooled, and processed[6] to give dispersed cell suspensions in Earle's saline buffered to *p*H 7·0 with ***tris***, and supplemented with 20 per cent normal rat serum. The cells were sedimented by gentle centrifugation, and washed three times to remove free soluble antibody. Single cells were then isolated in microdroplets by a simple modification[4,5] of de Fonbrune's oil chamber method[7]. This consisted essentially of depositing tiny droplets (volume 10^{-7}–10^{-6} ml.) on the surface of a coverslip and immersing them in paraffin oil. The coverslip was then inverted over a chamber filled with oil. The easiest method for preparing droplets containing one cell was to dispense a large number of droplets by free-hand manipulation from a suspension containing 1 : 400 by volume of lymph node cells. These droplets contained from nought to six cells; each droplet was later recorded for its cell content. Larger droplets containing up to 100 cells could also be prepared. Alternatively, droplets containing exactly one cell each could be prepared by micromanipulation, but this was more tedious, due to the adhesion of the cells to the micropipette. The oil chamber was then incubated at 37° C. for 4 hr. At the end of this time, the chamber

* Genetics Paper No. 685.

was placed on a microscope and the droplets surveyed at one hundred-fold magnification, dark ground. With a micropipette controlled by de Fonbrune micromanipulator, about ten bacteria were introduced into each droplet. Half the droplets were inoculated with *S. adelaide*, and the other half with *S. typhi*. After twenty minutes at room temperature, the droplets were observed for motility of the organisms. Total loss of motility of all the organisms was recorded as 'inhibition'. If even one organism in the droplet remained motile, this was recorded as 'no inhibition'. For control purposes, the suspending medium, the final supernatant from the washings, and the whole cell suspension prior to incubation were all shown to be free of inhibitory activity. Droplets prepared from the final cell suspension but containing no cells were also scored and found to lack inhibitory activity. Cells from several untreated rats were tested and these failed to elaborate a factor inhibiting the motility of the bacteria. Antisera against each serotype showed negligible cross-reaction with the other.

A proportion of the cells from immunized animals developed a factor immobilizing the test bacteria, and this was presumed to be antibody. All droplets containing single cells which were seen to immobilize the first serotype were then inoculated with about ten organisms from the second. After a further twenty minutes at room temperature, they were again observed for motility. The results of a typical experiment are recorded in Table 1. They indicate that none of the single cells was able to immobilize the organisms of both strains. To date 456 single cells have been tested for antibody produc-

Table 1. ANTIBODY PRODUCTION BY ISOLATED CELLS

No. of cells in drop	No. of drops inhibitory	No. of drops tested
First tested versus *S. adelaide*		
1	6*	39
2	5	25
3	7	24
4	6	21
5	6	10
6–10	17	33
First tested versus *S. typhi*		
1	3*	18
2	6	26
3	0	14
4	3	14
5	1	8
6–10	22	42

Lymph node cells from rats presensitized to *S. adelaide* plus *S. typhi* were dispensed in micro droplets and incubated for 4 hr. They were then tested by the introduction of motile bacteria.

* These droplets were also tested for activity against the alternative serotype and were negative.

tion, 228 against each of the two organisms. Out of these, 33 were active against *S. adelaide* and 29 against *S. typhi*, but none of the 62 immobilized both strains.

These results imply that when an animal is stimulated with two contrasting antigens, individual cells tend to form one species of antibody. We cannot exclude a residual production of other antibodies at lower rates. The experiments were provoked by current hypotheses on the role of clonal individuation in antibody formation[8,9], with which they are consistent so far as they go. However, further studies will be needed to determine whether the assortment of antibody-forming phenotypes reflects a genotypic restriction or whether it is more akin to such phenotypic effects as interference between related viruses, or diauxie and competition in enzyme formation.

We are indebted to Sir Macfarlane Burnet for his interest, encouragement and hospitality. This work was aided by a grant from the National Health and Medical Research Council, Canberra, Australia. It was done as part fulfilment of the requirements for the degree of doctor of philosophy in the University of Melbourne (G. J. V. N.).

G. J. V. NOSSAL
JOSHUA LEDERBERG*

Walter and Eliza Hall Institute
of Medical Research,
Melbourne.
March 25.

* Fulbright Visiting Professor of Bacteriology, University of Melbourne; from Department of Medical Genetics, University of Wisconsin, Madison.

[1] Fagreus, A., *J. Immunol.*, **58**, 1 (1948).
[2] Thorbecke, G. J., and Keuning, F. J., *J. Immunol.*, **70**, 129 (1953).
[3] Wesslen, T., *Acta Dermato-Venerol.*, **32**, 265 (1952).
[4] Lederberg, J., *Genetics*, **41**, 845 (1956).
[5] Lederberg, J., *J. Bacteriol.*, **68**, 258 (1954).
[6] Harris, S., Harris, T. N., and Farber, M. B., *J. Immunol.*, **72**, 148 (1954).
[7] De Fonbrune, P., "Technique de micromanipulation" (Masson, Paris, 1949).
[8] Burnet, F. M., *Austral. J. Sci.*, **20**, 67 (1957).
[9] Talmage, D. W., "Ann. Rev. Med.", **8**, 239 (1957).

Printed in Great Britain by Fisher, Knight & Co., Ltd., St. Albans.